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Species Composition and Seasonal Activity of Phlebotomine sand flies (Diptera: Psychodidae) in Paveh County, West of Iran

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ABSTRACT

Phlebotomine sand flies are biological vectors of leishmaniasis in Iran and many other countries. In order to determine fauna and seasonal activity of sand flies, an investigation was carried out in Paveh County, Kermanshah Province, west of Iran. Using sticky traps sand flies collected in 5 sites of Paveh County from May to October of 2015. Activity of sand flies started in late April and ended in early October. Totally 2110 (71.1% male and 28.9% female) phlebotominae were collected from outdoors (64.6%) and indoors (35.4%) respectively. Twelve species in two genera were identified including: *Phlebotomus alexandri, Ph. Sergenti, Ph. papatasi, Ph. major, Ph. tobbi, Ph. brevis, Sergentomyia sintoni, S. dentata, S. antennata, S. palestinensis, S. pawlowskyi and S. tiberiadis. Phlebotomus alexandri* was predominant species in the region with 50% all of collected sand flies and *Ph. brevis* comprised only 0.04% of specimens. Due to documented report of seropositive cases of visceral leishmaniasis in Paveh County and presence of proven vectors in this area, further studies on epidemiological and entomological aspects of disease are recommended.

Key words: Phlebotomine sand flies; Fauna; Leishmaniasis; Paveh, Iran

INTRODUCTION

Although the causal agent of the leishmaniasis has been known for more than 100 years and detailed descriptions of cutaneous form by some scientists such as Avicenna can be traced back many hundreds of years, leishmaniasis, still is an important endemic disease in numerous countries around the world [1,2].The leishmaniasis rank as the leading NTD (Neglected tropical diseases) in terms of mortality and morbidity with an estimated 50,000 deaths in 2010, which over two thirds of new CL cases occur in 6 countries: Afghanistan, Algeria, Brazil, Colombia, Iran (Islamic Republic of) and the Syrian Arab Republic [3,4].

Leishmaniasis are the most prevalent arthropod borne diseases in Iran and recorded cases of cutaneous form reach 20000 cases in the year, although the real number of affected patients is estimated to be 4 or 5 times more than this [5].

Because of the varied spectrum of clinical manifestations, leishmaniasis are often referred to as a group of diseases ranging from localized self-healing cutaneous to more severe or visceral form which generally involves the spleen, liver and bone marrow and in untreated cases may lead to death [4,5].

The disease is classified as a zoonotic infection so animals such as rodent and canine may play an important role as reservoir hosts [6]. The etiologic agents are obligate, intracellular protozoan of the genus leishmania and order *kinetoplastida*, which have single mitochondrion located near the basal body of the flagellum and can be seen as two different morphological forms: amastigote (without flagellum) and promastigote (with flagellum) [7-10]. Amastigotes are seen in humans and other mammalian macrophage cells, whereas, promastigotes are observed in sand flies and culture media [11].

Phlebotomine sand flies are the only proven natural vectors of *leishmania* species. There are more than 900 species and subspecies of sand flies, distributed almost on all continents except Antarctica [12].They are hematophagous insects, which 98 species are either proven or suspected vectors of human leishmaniasis [13].

Sand flies are also vectors of some other important viral and bacterial diseases including: Papatasi fevers

(three-day fevers), summer meningitis and bartonellosis [12,14,15].

Present study aimed to elucidate the diversity and seasonal activity of phlebotomine sandflies in Paveh County, which is located in the vicinity of Iraq and some known foci of leishmaniasis.

MATERIALS AND METHODS

This descriptive cross-sectional study was done on sand flies (leishmaniasis vectors) in selected sites in Paveh County located in west of Iran between 45°20′ E and 48° 1′E longitude and 33° 37′ N and 35°17′ N latitude in West Iran. The County is 120 km far from the center of Kermanshah Province and is bordered in north and east with Kurdestan Province, in south with Ravansar and Javanrud counties and in the west with Iraq (Fig. 1). Paveh has 800 km^2 area and 60000 residents according to 2015 census report. Weather of this county is generally temperate climate with semi-cold winters and mild summers.

Annual precipitation is around 700-750mm and August and January are the hottest and coldest months respectively. In this study, five sites were chosen in five different geographical and climatic parts of Paveh County (Table.1). These sites are Nowdesheh in the north, Hirwe in the west, Nooryab in the east, Desheh in the center and Bayengan in the southern region of the County. From the beginning of May till late October, in every field survey, 30 sticky traps were installed in indoor places such as bedrooms, sitting rooms, stables, bathrooms and toilets. Also 30 sticky traps were placed in outdoor places like rodent burrows, ruined houses cracks in walls and stone clefts and repeated every two weeks during the study period (Fig. 2).

Traps were installed before sunset in selected sites and collected before sunrise. Specimens were transferred to laboratory for identifying and species diagnosis. In the laboratory, sand flies were removed from the sticky traps by a fine dissection needle and after removing the oil with acetone the samples were transferred to 70% ethanol containers and kept in a cool place till slide preparation.

For identifying, first a slide prepared as below: one drop of puri's media was placed in the center of a slide and after cutting off the head and genitalia of individual sand flies, the sand fly was laid on slide and covered with cover slip [16].

Sand flies were identified using taxonomic keys and comparing with species of the standard collection to the extent possible according to the keys described by Nadim and Javadian, Lewis, Akhoundi and a pictorial key [17-20]. Females were identified based on the shape of their spermatheca, pharynx armature and cibarial teeth. Males were identified by the shape of their genitalia.

Fig. 1: The map of Kermanshah province and Paveh County location

Fig. 2: A sampling site in Paveh County (sticky traps in livestock places), 2015

Table1: Geographic locations of sampling sites in Paveh County, 2015

Situation / Place	Altitude (above sea level)	Longitude	Latitude
Hirwe	711 m	46°54′	35° 10′
Bayengan	1015 m	46° 16′	34° 58′
Desheh	1133 m	46° 11′′	35° 18′
Nooryab	1438 m	46° 43′	35° 47′
Nowdesheh	1480 m	46° 15′	35° 10′

RESULTS

During six month filed survey (from late May to late October), totally 2110 specimens were collected. The activity of sand flies in Paveh county starts in late May and after two activity peaks in late June and another in early August, ceased in late October (Fig. 2). As indicated in Table 2, twelve species distributing in two genera (six *Phlebotomus* and six *Sergentomyia*) were identified. From all captured specimens1820 specimens (86.3%) were

Phlebotomus and 290 specimens (13.7%) were *Sergentomia*. *Phlebotomus alexandri* with 1056 specimens (50%) was the most abundant species followed by *Ph. sergenti* with 301 specimens (14%). The other identified species were *Ph. papatasi*, *Ph. major*, *Ph. tobbi*, *Ph. brevis*, *Sergentomyia sintoni*, *S. dentata*, *S. antennata*, *S. palestinensis*, *S. pawlowskyi*, and *S. tiberiadis*. Also *S. sintoni* was dominant species in Sergentomia genus and *Ph. brevis* with 1 specimen (0.04%) was the least abundant species in genus *phlebotomus*. In all sampling of *phlebotomus* species, male dominance was recorded (average male/female ratio was 3.52:1) whereas in case of Sernentomyia species Male/Famale ratio was different and almost in all trapping the number of female was higher.

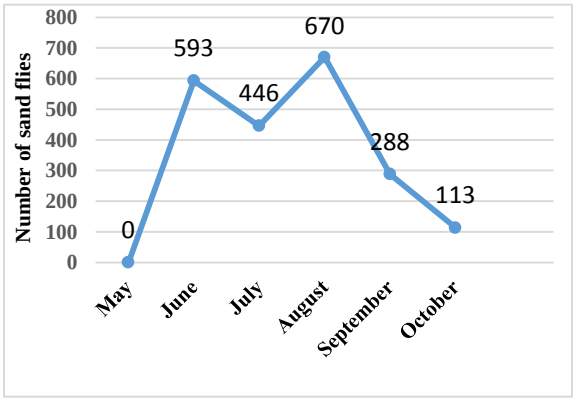

Fig. 2: Sand flies seasonal activities in the Paveh County, 2015

Table 2: Sand flies collected from Paveh County and their relative abundance

Species	Male	Female	Total	Percent	Sex Ratio
Ph. alexandri	884	172	1056	50	513
Ph. sergenti	237	64	301	14.3	370
Ph. papatasi	173	122	295	14	141
Ph. major	118	44	162	7.7	268
Ph. tobbi	5	-	5	0.23	-
Ph. brevis	1	-	1	0.04	-
S. sintoni	40	76	116	5.5	52
S. dentata	20	82	102	4.8	24
S. antennata	17	30	47	2.2	56
S. palestinensis	2	15	17	0.8	13
S. pawlowskyi	2	3	5	0.23	66
S. tiberiadis	1	2	3	0.14	50

The comparison of sandflies captured outdoors or indoors can be seen in Table 3. A total of 1362(64.6%) were captured outdoors and 748 (35.4%) sand flies were collected indoors. *Phlebotomus alexandri* and *Ph. sergenti* and all collected sergentomyia species were more abundant outdoors.

Distribution of sandflies species in different sites has been shown in Table 4. Hirwe site in west part of Paveh County with 826 specimens was the most populated site, whereas Nodesheh in north part of the County with only 197 specimens had the lowest number of sand flies.

Table 3: Number and percent of sand flies captured in indoors and outdoors in Paveh County, Kermanshah province, West of Iran

Species	Indoors	Percent	Outdoors	Percent	Number	Total
Ph. alexandri	338	45.2	718	52.7	1056	50
Ph. sergenti	137	18.3	164	12	301	14.3
Ph. papatasi	152	20.3	143	10.5	295	14
Ph. major	75	10	87	6.4	162	7.7
Ph. tobbi	3	0.4	2	0.1	5	0.23
Ph. brevis	1	0.1	-	-	1	0.04
S. sintoni	27	3.6	89	6.5	116	5.5
S. dentata	7	0.9	95	7	102	4.8
S. antennata	4	0.5	43	3.2	47	2.2
S. palestinensis	3	0.4	14	1	17	0.8
S. pawlowskyi	-	-	5	0.4	5	0.23
S. tiberiadis	1	0.1	2	0.1	3	0.14
Total	748	100	1362	100	2110	100

Table 4: Abundance of captured sand flies in different sites in Paveh County, 2015

Place / Species	Hirwe		Bayengan		Desheh		Nooryab		Nowdesheh		Total
	M	F	M	F	M	F	M	F	M	F	
Ph. alexandri	414	64	150	39	134	32	112	21	74	16	1056
Ph. sergenti	34	15	64	18	61	12	44	15	34	4	301
Ph. papatasi	35	26	23	22	63	36	35	23	17	15	295
Ph. major	15	4	40	14	37	17	21	8	5	1	162
Ph. tobbi	2	-	3	-	-	-	-	-	-	-	5
Ph. brevis	-	-	1	-	-	-	-	-	-	-	1
S. sintoni	28	44	2	15	-	-	4	15	6	2	116
S. dentata	13	50	-	3	-	-	4	25	3	4	102
S. antennata	7	15	-	-	-	-	5	11	5	4	47
S. palestinensis	-	7	-	-	-	-	-	3	2	5	17
S. pawlowskyi	-	-	2	-	-	-	-	3	-	-	5
S. tiberiadis	1	2	-	-	-	-	-	-	-	-	3
Total	549	227	285	111	295	97	225	124	146	51	2110

DISCUSSION

Despite scientific struggles and financial supports to reduce the burden of the disease in last decades, continues conflict in many part of the world and related human migration and also lack of an effective vaccine, emergence of drug resistance, urbanization and some types of immunodeficiency disorders, caused leshmaniasis continues to be an important health problem.

During the present study, totally 2110 sand flies were collected, more than 86.3% of them belonging to the genus Phlebotomus.

Five species including: *Phlebotomus. alexandri*, *Ph. Sergenti*, *Ph. papatasi*, *Ph. major* and *Ph. tobbi*, are known or suspected vectors of different types of leishmaniasis in Iran and other parts of old world [21-23].

Due to results presented in Fig.2 monthly activity of sand flies in Paveh County starts at late May and after two activity peaks in late June and early August, ends in late October. It seems that there is two generations of sand flies in Paveh and second generation has a higher population.

Similar activity pattern has been reported from Baft county in Kerman province, south-east of Iran, which the activity of sand flies started in late May and terminated in late October with two activity peaks in early July and another in early August [24]. Both Baft and Paveh are situated in a mountainous area.

According to previous studies sand flies activity initiation depends on climatic factors such as ambient temperature and relative humidity [25]. So milder climate can increase the duration of activity season as can be seen in a study which has been done in Bushehr County, southern Iran, in which the activity of sand flies started in early April and terminated in early January and their highest activity occurred in early July [26]. This prolonged activity period can also be correlated with warmer climatic conditions in southern areas of Iran. So it is clear that the active period of sand flies is more prolonged in warmer regions of studied areas of Iran.

As the Table 2 shows during this study 12 different species have been identified. This species diversity might be due to different geographical and climatic conditions and also a suitable environment for growth and development for sand flies in Paveh County. For example, Nowdesheh and Nooryab are situated in relatively higher altitude (1480 m and 1438 m above sea level respectively) with cold winters and mild summers, whereas Hirwe is located in lower altitude (711 m above sea level) and so has relatively higher temperature. It is in accordance with some studies which reveal climatic conditions may be one important factor for sand fly species diversity [27]. The effects of environmental factors on diversity and species distribution of phlebotomine sand flies also can be seen in similar studies which have been carried out in nearby areas. For example, although the results of the present study show that *Ph. alexandri* comprise 50% of collected phlebotomine, during recent studies performed in the Ravansar County, which is located in south east of Paveh County the predominant species reported to be *Ph. papatasi* with 51.2% of all collected sand flies [28].

Hirwe site in west of Paveh County with 826 specimens was the most populated site, whereas Nodesheh in north of the county with only 197 specimens was the was the least populated sand fly capture collection site, which in this case lower population of sand flies in Nodesheh may be related to lower ambient temperature in this area.

Higher population of vectors may also provide more chance for contact with hosts and leads to diseases transmission, especially in presence of reservoir hosts which have been reported in Paveh and adjacent areas [29, 30]. Also as the Table 4 shows, areas with more populated phlebotomine have more diverse species.

In our study, *Ph. alexanderi* was the dominant species in both indoor and outdoor places. This species has more tendency toward mountainous regions rather than plains, although it has been reported in both mountains and plains [25]. *Phlebotomus alexandri* is considered as the vector of visceral leishmaniasis in Iran and some other countries [24, 31]. The dominance of this species in combination with recent reports of presence of seropositive cases of visceral leishmaniasis in Paveh is alarming and needs more attention [29]. *Phlebotomus sergenti* and *Ph. papatasi*, which were second and third regarding their abundance, are considered as definite vectors for cutaneous leishmaniasis in urban and rural regions of Iran respectively [32,33].

In our study, results from sex ratio have demonstrated a significant difference between males and females, with 1500 specimens (71.1%) males, and 610 specimens (28.9%) females. Sex ratio for two dominant species, *Ph. alexandri* and *Ph. sergenti* was 514 and 317 males per 100 females respectively. In a study in Hormozgan province, southern Iran, the sex ratio for Ph. alexandri as dominant species were 312 males per 100 females [34]. In another study in Khash County, Sistan and Balutchestan Province, south-east of Iran, 74.77% of specimens were male and 25.23% were female [35]. In other studies which have been conducted in different regions in Iran, using sticky trap method, the number of males always was higher than female [36-38]. So it seems that the collection method is important in Male/ Felale ratio [21].

In this research, 13.7% of specimens were Sergentomyia and dominant species was *S. sintoni*, which is considered as the vector of lizard leishmaniasis although it does not have any role in disease transmission to humans [25, 39].

CONCLUSION

Present study has provided some essential data on species composition and seasonal activity of phlebotomine sand flies in Paveh. Presence of some proven vectors of leishmaniasis, Proximity of Paveh County to Iraqi border, prolonged political instability in this country, which causes the refugee problem, illegal immigration through Paveh toward inside parts of Iran, and presence of seropositive cases in Paveh may lead to an increase in leishmaniasis cases in region. Moreover introducing the disease to new areas may leads to appearance of more virulent strains of pathogen and since there is no vaccine to prevent leishmaniasis, people and health workers must be informed about importance of this disease and its transmission pathways [29, 30, 40]. Also further studies on epidemiological and entomological aspects of disease are recommended.

ETHICAL ISSUES

This study was approved by the Hamadan University of Medical Sciences ethical committee and instruction given by committee was followed regarding experiments throughout the study.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

AUTHORS' CONTRIBUTION

CM and AS conceptualized and designed the study, identified the samples and drafted the manuscript. SRJ analysed data, SM helped with sample collection. AS, AHZ and BD supervised the project. All authors discussed the results and implications and commented on the manuscript at all stages.

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